STUDIES IN ARTIFICIAL PARTHENOGENESIS.

V. The Anomalous Action of Mercuric Chloride.

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Traube[1] in 1909 made the claim that substances of low surface tension were more effective in producing parthenogenesis than those of higher surface tension. In 1913 and again in 1915, Heilbrunn[2] proposed a theory of membrane elevation in the sea-urchin egg, according to which the lifting off of the membrane (in this egg) is the direct result of a lowering of its surface tension. If this surface tension theory of membrane elevation is correct, only those substances which cause a decrease in surface tension can produce typical membrane elevation. So far as previous experiments have gone, this is true. There is no case in which membrane elevation has been produced by an agent which does not sharply lower surface tension (Heilbrunn, '24).

During the past summer an apparent exception was discovered. In the course of some work which we were doing with dilute solutions of mercuric chloride in sea-water, my assistant Mr. D. E. S. Brown called my attention to what was apparently true membrane elevation in the egg of the sea-urchin *Arbacia*. Beautiful wide membranes arose from the egg surface. That these were truly elevated membranes (not swollen membranes) was shown by the fact that they could be made to collapse on the addition of egg albumin to the sea-water.

The parthenogenetic action of mercuric chloride was known to F. R. Lillie and he mentions it briefly in a paper published in 1921[3] (see p. 140).

It seems certain that mercuric chloride as such could have no great effect on surface tension. Apparently, therefore, the fact that the mercury salt causes membrane elevation can not be made to fit in with the surface tension theory. Because of this apparent conflict, it was thought worth while to further investigate the action of the mercuric chloride, with a view to the possible abandonment of the surface tension theory. Although many experiments were performed, only a few of them will be considered and these rather hastily. They suffice to show that the action of mercuric chloride is not a simple one.

If an *m*/10 solution of mercuric chloride is diluted a thousand times with sea-water, then an "*m*/10,000 solution of mercuric chloride in sea-water" is obtained. A solution of this sort is very favorable to membrane elevation. Solutions decidedly more concentrated than *m*/10,000 do not act as well. In an *m*/1000 solution, the membranes, if they lift off at all, become only partially elevated, apparently they stick to several points of the egg surface as they attempt to lift away from it. On the other hand extreme dilutions are also ineffective, although it is possible to obtain good membrane elevation in solutions as weak as *m*/100,000. Thus there is a wide range of concentration in which the mercuric chloride is effective. Most of the experiments were made with an *m*/10,000 solution (in sea-water).

On exposure to the mercuric chloride, membrane elevation does not occur immediately. After 3 or 4 or 5 minutes, the membranes can be seen rising from the surface of the eggs. The effect of the sublimate is not solely a cortical effect. After eggs have been exposed for about 12 minutes other changes may begin to appear. In many cases the cell undergoes what is apparently a cell division. At any rate it becomes constricted. Usually this constriction divides the egg unequally, a large cell and a small cell are formed. In such cases of unequal division the smaller cell usually contains a mass of pigment and the larger cell is comparatively colorless. Now and then instead of an unequal division there is a furrow around the very center of the egg with an accumulation of pigment granules underneath the furrow. No attempt was made to discover whether nuclear changes accompanied the changes in the cytoplasm. If the constrictions of the cytoplasm do represent anything at all comparable to a normal cell division, it is surprising that they occur so soon after the eggs are exposed to the mercuric chloride. Typically the Arbacia egg does not divide until 50 or 60 minutes have elapsed after fertilization. The cytoplasmic constrictions just described make their appearance 12 or 15 minutes after the eggs are placed in the mercury solution. The constriction of the cytoplasm is not followed by anything approaching normal development, even if the egg is removed from the solution of mercuric chloride and placed in normal sea-water.

Ordinarily when Arbacia eggs are exposed to an *m*/10,000 solution of mercuric chloride in sea-water, only about 10 per cent. of the eggs undergo membrane elevation. Why 10 per cent. of the eggs should throw off perfect membranes and the other 90 per cent. should show no change at all, was for a time a mystery.

After a number of experiments under diverse conditions, it was found that the percentage of eggs undergoing membrane elevation could be greatly increased by allowing the eggs to age. If eggs are taken from the ovary and placed directly into dilute solutions of mercuric chloride, no membrane elevation occurs. On the other hand if they are allowed to lie in sea-water in shallow dishes for 5 or 6 hours, practically a hundred per cent. will undergo membrane elevation when exposed to the mercuric chloride. In working with this reagent it is therefore advisable to allow eggs to age before starting to experiment with them.

The effect of aging is shown in the following table. The first column of this table gives the number of minutes the eggs were allowed to remain in sea-water before they were treated with *m*/10.000 mercuric chloride. Only the eggs of a single female were used but these were very plentiful. On removal from the ovary they were placed in 50 cc. of sea-water in a fingerbowl. After a time, as a result of the constant removal of material, the eggs at the bottom of the fingerbowl were no longer evenly distributed, some being more closely clustered than others. In the denser masses of eggs, the aging process apparently proceeded at a slower rate and this accounts for the irregularities in the table. The second column shows the percentage of eggs undergoing membrane elevation when subjected to mercuric chloride. To obtain this percentage two hundred eggs were counted in every instance except in the case of the eggs aged for 268 minutes. Only a hundred of these were counted.

Time Eggs Remained in Sea-water before Treatment with $HgCl_2$, Minutes.	Percentage of Eggs Showing Membrane Elevation.
0	0
5	6½
10	7½
15	10
20	10
30	11½
40	13
50	14½
60	17
81	17½
103	17½
128	15½
158	16
193	18½
238	30½
268	21
300	62½
345	94½

Although the table is not as regular as might be wished, it is clear that as the eggs lie in sea-water they become increasingly susceptible to mercuric chloride. This fact is interesting for at least two reasons. In the first place it apparently does not harmonize very well with the fertilizin theory of fertilization as developed by F. R. Lillie.[3] Aging of eggs, according to this theory, involves a loss of fertilizin, a material which is regarded as essential to fertilization, and yet in the experiment described above, such aging favored a cortical change characteristic of fertilization. But the case of mercuric chloride is certainly a special one, and it is doubtful if any general conclusions for or against the fertilizin theory can be derived from it. This will be more obvious from the later discussion.

Our main interest is elsewhere. Why do eggs after standing in sea-water respond more readily to treatment with mercuric chloride? Possibly in or around eggs fresh from the ovary there is some substance present which exerts an interference. This substance escapes on standing; it may be gaseous. On a priori grounds one would suspect carbon dioxide.

Experimental evidence favors this conjecture. When eggs were allowed to stand until they became highly susceptible to mercuric chloride and were then treated with a solution of the sublimate in sea-water which contained a trace of hydrochloric acid, they did not lift off membranes. If now the solution of mercuric chloride in acidified sea-water was shaken vigorously for a few minutes it again became potent as a stimulant to membrane elevation. In one experiment 4 cc. of $n/10$ HCl was added to 200 cc. of sea-water. To this was added 11 cc. of $m/1000$ $HgCl_2$. The resultant solution had a p_H below 6.8 and caused no membrane elevation. It was shaken until the p_H rose to above 7, but it still remained ineffective. It was then shaken vigorously for several minutes, and the p_H rose well above 7. After such shaking it produced membrane elevation generally. The effect of the shaking is to remove carbon dioxide from the solution. It seems certain from this experiment that the presence of carbon dioxide interferes with the production of membrane elevation by mercuric chloride.

Our first point then is the fact that mercuric chloride causes membrane elevation only in the absence of any considerable quantity of carbon dioxide.

A second point, which was not investigated as closely as it might have been, is the fact that sublimate treated eggs, if centrifuged one or two minutes after the exposure begins, do not lift off membranes. In one experiment no membranes were lifted off when eggs were centrifuged either one or two minutes after they had been placed in a $m/10,000$ $HgCl_2$ solution. Those centrifuged $3\frac{1}{2}$ minutes after being placed in the solution showed membrane elevation in 91 out of 100 cases. The centrifugal force used was 4,968 times gravity and in this experiment the eggs were centrifuged for 40 seconds in each instance. Apparently it is not necessary to centrifuge the eggs for as long a time as 40 seconds. In other experiments it was found that centrifugal treatment for only 5 seconds was enough to prevent all but a small percentage of the eggs from undergoing membrane elevation. The reason for the effect of the centrifuge is not certain. A plausible hypothesis, but by no means the only one, is that the mercuric chloride reacts with the jelly of the egg to form mercurous chloride and chlorine, and that it is the chlorine which is important for membrane elevation. Centrifuging removes the jelly from proximity to the egg surface. In favor of this view is the fact that mercuric chloride regularly does break down into calomel (or mercuric oxychloride) and chlorine in the presence of organic substances (Mellor,[4] Gmelin-Kraut [5]). On the other hand it should be pointed out that if the jelly is removed from the eggs either by shaking or centrifuging or both, and the eggs are then subjected to mercuric chloride solution, they undergo membrane elevation just as well as when the jelly is present. This fact need not interfere with our hypothesis. We can assume that although ordinarily the mercuric chloride reacts with the jelly to form chlorine, in the absence of chlorine it may react with the cortex of the egg itself.

A test of this point might be made by removing the jelly, treating with mercuric chloride and then centrifuging. If the above interpretation is correct then centrifuging of jelly-free eggs should not interfere with membrane elevation produced by the mercury salt. Unfortunately such a test was not made.

But there is other evidence in favor of the view that mercuric chloride reacts with some part of the egg. Sublimate solutions lose their effectiveness when allowed to stand over eggs. In one instance, after an $m/10,000$ solution had stood over eggs for 10 hours it in large measure lost its effectiveness as a stimulant to membrane elevation. Although a control of the same solution produced typical membrane elevation, the solution which had stood over eggs caused at best only a partial and slight membrane elevation. Even in the absence of eggs an $m/10,000$ mercuric chloride solution slowly loses its power to cause membrane elevation. The loss of this power is much more rapid in the presence of eggs.

If we assume that mercuric chloride gives off chlorine and it is this substance which causes membrane elevation, it should be possible to duplicate the action of the mercury salt with chlorine itself.

Chlorine gas was manufactured by adding hydrochloric acid to potassium permanganate and the gas was then passed through sea-water. If two drops of the solution of chlorine in sea-water are added to 20 cc. of sea-water the resultant solution is then effective in inducing membrane elevation. The results with chlorine itself are not as good as those with mercuric chloride. It is hard to regulate the amount of chlorine and only a very narrow range of concentrations may be used. One drop more or less of chlorine water added to 20 cc. of sea-water would often determine whether the resultant solution would be effective or not. Moreover the number of eggs introduced into the chlorine solution was an important factor. Thus in one experiment two stender dishes were each filled with 20 cc. of sea-water plus 2 drops of chlorine water. Many eggs were placed in one of the dishes, only a few in the other. In the latter case 78 per cent. of the eggs underwent partial or complete membrane elevation; in the dish containing many eggs no membrane elevation occurred at all.

It is easy to suppose that the addition of mercuric chloride to eggs furnishes a mechanism for supplying approximately the proper dose of chlorine to the egg surface. This is probably the reason that solutions of chlorine alone are not as effective as the mercuric chloride.

An argument that might be advanced against the idea that the action of mercuric chloride is due to chlorine is the fact that mercuric nitrate is also effective in causing membrane elevation. In order to exclude the presence of chlorine, the mercuric nitrate was made up in a 0.9 *m* sugar solution. A solution roughly *m*/10,000 was prepared. The action of such a solution is very peculiar. Extremely wide membranes are lifted off, membranes which are quite different in appearance from the normal-looking ones produced by mercuric chloride. It would be easy to show that a reaction occurs between the sugar and the mercuric nitrate, but no effort was made to determine the products of such a reaction. However it is believed that one of the reaction products causes the membrane elevation.

From the evidence cited it is obvious that the action of mercuric chloride is complex. It appears certain that the reagent does not act in its original form but undergoes some sort of a chemical transformation. There is good reason to believe that chlorine is produced, and that this substance acts on the eggs and incites membrane elevation.

If this view is correct then the fact that mercuric chloride solutions are effective in causing membrane elevation can not be used as an argument against the surface tension theory. If the sublimate forms chlorine we know that a substance of extremely low surface tension must be present.

It is possible that many more facts with regard to the action of mercuric chloride on egg cells or cells in general might easily be obtained by a further study of the sea-urchin egg in the presence of this reagent. Our only interest in the subject lay in the possibility of overthrowing the surface tension theory of membrane elevation. As soon as it was evident that no such overthrow was possible on the basis of the mercury evidence, no additional experiments were planned.

Summary.

1. Dilute solutions of mercuric chloride in sea-water cause typical membrane elevation in the sea-urchin egg in spite of the fact that they presumably do not lower surface tension.

2. The action of the mercuric chloride is favored by aging the eggs. Eggs fresh from the ovary are not acted upon, and the percentage of membrane elevation on treatment with the sublimate solution increases in proportion to the time the eggs have stood in sea-water before being subjected to the reagent.

3. The favorable effect of aging is apparently due to the removal of carbon dioxide. The addition of carbon dioxide prevents membrane elevation by mercuric chloride.

4. If eggs are centrifuged one or two minutes after the treatment with mercuric chloride is begun, membrane elevation is generally prevented.

5. Solutions of mercuric chloride in contact with eggs lose their power of provoking membrane elevation.

6. The facts cited in 4 and 5 are regarded as evidence in favor of the view that mercuric chloride reacts with the jelly or cortex of the egg to form chlorine. Such a reaction is in accord with the usual behavior of mercuric chloride in the presence of organic materials.

7. Chlorine gas is effective in producing membrane elevation.

8. The action of mercuric chloride in causing membrane eleva-

tion is probably due to the formation of chlorine. Since chlorine has a very low surface tension, the fact that mercuric chloride causes membrane elevation can not be used as an argument against the surface tension theory.

REFERENCES.

1. **Traube.**
'09 Biochem. Zeitsch., XVI., 182.

2. **Heilbrunn.**
'13 Biol. Bull., XXIV., 343; 1915, *ib.*, XXIX., 149; 1924, *ib.*, XLVI., 277.

3. **Lillie.**
'21 Biol. Bull., XLI., 125.

4. **Lillie.**
'19 Problems of Fertilization. Chicago, 1919.

5. **Mellor.**
A Comparative Treatise on Inorganic and Theoretical Chemistry, Vol. IV., London, 1923.

6. **Gmelin-Kraut.**
'14 Handbuch der anorganischen Chemie. Bd. V., Abt. 2. 7te Aufl. Heidelberg, 1914.